

A P P E N D I X

Marked-up Version of Changes

IN THE SPECIFICATION:

Page 1, after the title and before line 3,

"This is a divisional of Application No. 09/367,223 filed January 10, 1995, the disclosure of which is incorporated hereby by reference.", has been deleted, and the following has been inserted therefor

-- This application is a Divisional of U.S. Appln. No. 09/559,418, filed April 26, 2000 (now allowed); which in turn is a Divisional of U.S. Appln. No. 08/367,223, filed January 10, 1995 (now U.S. Patent 6,228,358); which in turn is a § 371 of PCT/JP94/00753, filed May 9, 1994. The disclosure of each of which is incorporated herein, in their entirety, by reference. --

Page 2, lines 1-22 are changed as follows:

"By the way, active oxygen is important as a biological protection factor such as bactericidal action of [leucocyte] leucocytes, but it becomes apparent that the excessive production of active oxygen in the living body causes various tissue disorders.

As the ordinary [factor] factors for generating active oxygen, there have been known stresses, alcohols, peroxides, drugs, exercises and the like. It is pointed out that active oxygen and lipoperoxide generated by these factors are closely connected with cerebral nerve diseases, circulatory diseases, cancers, alimentary diseases, hepatic diseases, arterial sclerosis, renal diseases, diabetes, [ageing] aging and the like.

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As the ordinary [factor] factors for generating active oxygen, there have been known stresses, alcohols, peroxides, drugs, exercises and the like. It is pointed out that active oxygen and lipoperoxide generated by these factors are closely connected with cerebral nerve diseases, circulatory diseases, cancers, alimentary diseases, hepatic diseases, arterial sclerosis, renal diseases, diabetes, [ageing] aging and the like.

The living body retains a series of oxidation protection systems so [as] to protect itself against oxygen toxicity. On the other hand, in order to permit these systems to function normally, it is important to ingest oxidation nutrient components sufficiently. As the natural oxidation nutrient [component] components, there have been known vitamin E, vitamin C, s-carotene, polyphenol and trace elements (e.g. selenium, copper, zinc, etc.). For the purpose of affording an antioxidation action, food products containing these nutrient components have been developed."

Page 2, line 23-Page 3, line 7, are changed as follows:

"In vivo antioxidation mechanism is classified roughly into a preventive antioxidation action (controlling the generation of a radical) and a linkage-breakage type antioxidation action (scavenging and eliminating a radical which has already been generated) according to its action. Examples of those which have the former action include enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and the like. Examples of those which [has] have the latter action include the above antioxidation nutrient components."

Page 3, lines 12-18, are changed as follows:

"It is an object of the present invention to provide an antioxidation food product, an antioxidation preparation and an antioxidation method for the linkage-elimination of superoxide and hydrogen peroxide, which express a superoxide dismutase (hereinafter referred to as "SOD")-like activity and a catalase (hereinafter referred to as "CAT") activity, simultaneously, and which are particularly superior in preventive antioxidation action."

Page 3, lines 19-25 are changed as follows:

"The antioxidation food product of the present invention has an antioxidation action in the living body [including the interior of a digestive tract], comprising a fermented product produced by adding a manganese-containing natural material and fermenting with bacteria having a CAT activity. By "living body" is meant the physical body of living, multicellular organisms including, but not limited to humans and other mammals."

Page 4, lines 1-19, are changed as follows:

"That is, the present inventors have obtained a knowledge that specific bacteria among various lactic acid bacteria express no CAT activity under the environment in which manganese is not present, but if manganese is present in the growing environment, they incorporate manganese into their cells to express a Mn-CAT activity and a SOD-like activity, simultaneously[, and the present invention has been completed]. The antioxidation food product of the present invention is particularly suitable as a preventive antioxidation food product.

It is known that Mn-CAT is not affected by various inhibitors, modifiers, chelating agents, etc. in comparison with heme-CAT containing iron, and exhibits stability within a wide range of pH and [a] temperature.

Further, the antioxidation food product of the present invention may be a dried product, preferably a freeze-dried product which contains bacteria having a CAT activity and a manganese-containing natural material, in addition to the fermented product as described above by "manganese-containing natural material" is meant any material whether naturally

occurring or synthetically produced which contains the element manganese bound to another organic or inorganic chemical moiety. The term is not meant to include elemental manganese."

Page 4, line 25-Page 5, line 10, are changed as follows:

"Furthermore, according to the present invention, there is provided an antioxidation method in the living body including the interior of a digestive tract, which comprises ingesting a fermented product produced by adding a manganese-containing natural material to a food product and fermenting with bacteria having a CAT activity to express a SOD-like activity and a CAT activity, simultaneously.

BRIEF EXPLANATION OF THE INVENTION

Fig. 1 is a graph illustrating a change in SOD-like activity and CAT activity due to the addition of $MnCl_2$ or [that of] tea."

Page 7, lines 4-14, are changed as follows:

"Recently, the clinical application of SOD against 5 rheumatism, cardiac ischemia, etc. have been proceeded. However, there is a serious problem that the local amount of hydrogen peroxide is increased when SOD is used alone and, further, a hydroxy radical ($\cdot OH$) having a highest radical reactivity is formed. Accordingly, it is important to eliminate O_2^- and remove hydrogen peroxide by using CAT and GSH-Px in combination when employing SOD. Therefore, [according to] for the above reason, the coupling action between SOD-like and CAT obtained by adding Mn to *Lactobacillus plantarum* is considered to be very important."

Page 8, lines 9-22, are changed as follows:

"The manganese-containing natural material in the present invention is used for supplying manganese to the bacteria cells.

It is impossible to add manganese as an inorganic compound because manganese itself is not accepted as a food additive and, therefore, the natural material was used. Examples of the natural material containing a large amount of manganese include plants such as teas, vegetables (e.g. represented by cabbage, spinach, etc.), herbs and the like. Accordingly, it is important to add these plants so as to supply manganese which is necessary for expressing a CAT activity and a SOD-like activity to bacteria having a CAT activity. Particularly, since the tea contains a lot of antioxidation components such as catechin, vitamin C, various trace elements, etc., [high addition effect] additional effects can be obtained by taking advantage of the presence of an activity of these anti-oxidant components."

Page 8, line 23-Page 9, line 5, are changed as follows:

"Particularly, the addition of the tea to *Lactobacillus plantarum* [cause] causes the following effect, that is, not only the SOD-like activity and CAT activity are expressed but also the SOD-like activity is extremely high in comparison with the case using an inorganic Mn compound (e.g. manganese chloride $MnCl_2$, etc.) and, further, the SOD-like activity and CAT activity under gastric juice exposure can be maintained for a long period of time."

Page 9, lines 6-21, are changed as follows:

"It is preferred that the tea or the other natural material is added to a food product in the form of powder. [because the] The incorporation of manganese due to bacteria [becomes easy] is facilitated if the magnesium source is a powder. The pulverization is conducted by extracting a natural material with water and/or water-miscible organic solvent (e.g. alcohols such as ethyl alcohol, etc.) and then with an organic solvent which

is in-miscible to water (e.g. chloroform, ethyl acetate, butanol, etc.) to separate into an organic phase and an aqueous phase, recovering a dissolved solid content from the aqueous phase, followed by drying. Further, a solid content may also be recovered from a solution extracted with water and/or water-miscible organic solvent and then pulverized. Furthermore, an aqueous solution prepared by extracting the natural material with water may be added [as it is] without pulverizing, or [that which obtained by pulverizing,] the pulverized product of the natural material [finely] may be added as it is."

Page 10, lines 11-13, are changed as follows:

"Typical examples of the form of the antioxidation food product of the present invention include fermented food products [product] and (freeze)dried food products [product], as described above."

Page 11, lines 6-23, are changed as follows:

"Further, [the] a lactic acid bacteria beverage can be obtained by adding a predetermined amount of a manganese-containing natural material to a mixed solution of skim milk and sugar (e.g. glucose, sucrose, etc.), inoculating the mixed solution with lactic acid bacteria having a CAT activity and fermenting at 35 to 37 °C for about 12 to 72 hours. Yogurt (e.g. liquid yogurt type, juice type, etc.) can be produced according to a proportion of skim milk to a diluting solution (e.g. water, fruit juice, etc.). As the base to be fermented, for example, there can be used milk serum, low fat milk, etc., in addition to skim milk. Examples of the diluting solution include flesh, lactocoffee, etc., in addition to water.

The dried product in the present invention is obtained by mixing about 5×10^8 to 5×10^{10} bacteria cells 20 with 2 to 4 g of a manganese-containing natural material, adding an excipient to the mixture, followed by drying. Examples of the excipient include lactose, glucose, sucrose, [origosaccharide] oligosaccharide and the like."

Page 13, lines 4-8, are changed as follows:

"As shown in Fig. 1, the SOD-like activity increased in proportion to the amount of Mn to be added in both cases (addition of $MnCl_2$ /tea), but the absolute value of the SOD-like activity [in case of the addition of tea was well above (about 35 times when 200 UM of Mn is added)] is about 35 times greater when tea is added than when 200 M of the Mn is added."

Page 13, lines 14-15, are changed as follows:

"[By the way, the] The function of SOD and CAT is the elimination of O_2^- and H_2O_2 . However, SOD and CAT are enzymes and, therefore, it is said that SOD and CAT are devitalized even if they are orally ingested and no effect is obtained. However, since the SOD activity and CAT activity are retained in the bacteria cells in case of the Mn-added product produced by fermenting with *Lactobacillus plantarum*, it is expected that these activities are retained until the bacteria are killed. Further, as described above, since Mn itself and the component in the tea have a SOD-like activity but they are not enzymes, it is considered that they are not easily devitalized."

Page 14, lines 11-20, are changed as follows:

"Immediately after ligating [a pylorous] the pylorus of SD male rats (weight: 250 g), 5 ml of a product produced by fermenting with *Lactobacillus plantarum* was administered using an oral probe and contents in the stomach were recovered with

time to examine a change in SOD-like activity and CAT activity. The results are shown in Figs. 2 and 3. Further, a test product was obtained by adding MnCl_2 or a tea (powdered green tea) in the concentration (concentration of Mn) of $50 \mu\text{M}$ to an APT broth and, after inoculating with *Lactobacillus plantarum*, fermenting for 16 hours."

Page 19, lines 11-17, are changed as follows:

"As the product produced by fermenting with *Lactobacillus plantarum*, [those which] were obtained by adding [MgCl_2] MnCl_2 or a tea extract solution (those obtained by extracting 100 g of a green tea with 1 liter of hot water) to an APT broth in the concentration (concentration of Mn) of $50 \mu\text{M}$ and, after inoculating with *Lactobacillus plantarum*, fermenting for 16 hours."

Page 22, line 20-Page 23, line 6, are changed as follows:

"It has been known that the neutrophil [products] produces active oxygen so as to [detoxicate] detoxify foreign materials and bacteria in the living body. Particularly, the neutrophil has a dimutated system of hydrogen peroxide which is referred to as "MPO", thereby producing perchloric acid or monochlor amine having high cytotoxicity. At the time of the excessive reaction, the transudation of [MPO] the neutrophil to the exterior of [MPO] the neutrophil is confirmed and it is considered to be one factor to cause the disorder of the biomembrane. There is also a report that the infiltration of neutrophil is connected with its crisis mechanism in indomethacin-induced gastric ulcer and hydrogen peroxide-induced gastric mucosal lesions."

IN THE CLAIMS:

Claims 1-6 are being cancelled.

New Claims 7-10 are being added.

IN THE ABSTRACT:

Please delete the present Abstract, and insert therefor the substitute Abstract attached hereto.